REVIEW

The Role of Methane in Intestinal Diseases

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The volume of human intestinal gas is about 200 ml, and it is derived from complex physiological processes including swallowed air, diffusion from bloodstream into the lumen, and particularly intraluminal production by chemical reactions and bacterial fermentation. Gas is continuously removed by eructation, anal evacuation, absorption through the intestinal mucosa, and bacterial consumption. More than 99% of it is composed of hydrogen, oxygen, carbon dioxide, nitrogen, and other odoriferous gases. Methane (CH_4) production is detectable in about one third of healthy adult individuals. In the past years, several studies have been focused on CH_4 metabolism at the intestinal level and on the putative association between this gas and the pathophysiology of organic and functional bowel disorders. An overview of the present knowledge about the physiology of CH_4 metabolism and its role in intestinal diseases is provided in this report.

Am J Gastroenterol advance online publication, 9 March 2010; doi:10.1038/ajg.2009.744

INTRODUCTION

The human gastrointestinal tract harbors a complex microflora, which outnumbers the eukaryotic host cells by first order of magnitude (1). The intestinal microflora includes about 500 different bacterial species with a high concentration in the colon and is responsible for important functions such as immunoregulation and stimulation, the barrier effect, the metabolic and trophic function, and vitamin synthesis (**Figure 1**) (2). The regional differences in the gut microflora and the balance of single species are readily influenced by several factors such as luminal pH, bile concentration, motility, the host immune system (3); predominantly, by different available substrates, changes in colonic content and complex interrelationships ranging from commensalism to competition (4). In particular, the microflora degrades and ferments substrates that have either escaped digestion in the upper intestinal tract or have been produced by the host as short chain fatty acids and gases (**Figure 2**) (5).

Hydrogen (H_2) is formed by a variety of hydrolytic and saccharolytic bacteria as a mean of disposing or reducing equivalents from the anaerobic colonic environment (6,7). In humans, part of H_2 formed during colonic fermentation is excreted in breath and flatus where it is readily detected. However, interspecies H_2-transfer is now assumed to be the main process of H_2 disposal in the colon (7). Several mechanisms of H_2 utilization have been reported in the human large intestine including methanogenesis, dissimilating sulfate reduction, and acetogenesis. The latter process corresponds to the reduction of two moles of carbon dioxide (CO_2) by four moles of H_2 to form one mole of acetate (8–10).

Methane (CH_4) production consumes 4 mol of H_2 to reduce 1 mol of CO_2 to CH_4, a process that greatly decreases colonic gas volume. In contrast to H_2, CH_4 concentration remains relatively constant during the day, and does not depend directly on the availability of fermentable substrates present in the diet (11,12). H_2 breath test (HBT) is commonly used in clinical practice for the diagnosis of carbohydrate malabsorption, as the concentration of breath H_2 parallels the intestinal production from fermented carbohydrates (13,14). The clinical use of CH_4 measurement is still controversial (15,16).

In fact, the relation between H_2 and CH_4 production has been indeed considered a possible confounding factor in the interpretation of HBT. However, it has been reported that evaluation of breath CH_4 might enhance HBT accuracy. Despite these discrepancies, CH_4 production is usually disregarded in the interpretation of HBT and, in most instances, only H_2 excretion is measured (17–19).

METHANOBREVIBACTER SMITHII

As we know, the major products of human large intestine microbial fermentation are acetic, propionic, and butyric acids, H_2, CO_2, and CH_4 (20). Detectable CH_4 production, using breath-CH_4 analysis occurs in about one third of the adult population (21). Despite the absence of CH_4 in the breath of many subjects, methanogenic bacteria can be cultured from feces in the majority of them. Weaver, Miller, and Wolin's group suggested that CH_4 appears in breath only when the numbers of methanogenic bacteria reach a critical level, about 10^8/g dry weight contents. Therefore, dividing a population into producers and non-producers may be artefactual as everyone is a potential producer (14,22–24). Most likely, the

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Received 5 May 2009; accepted 15 December 2009

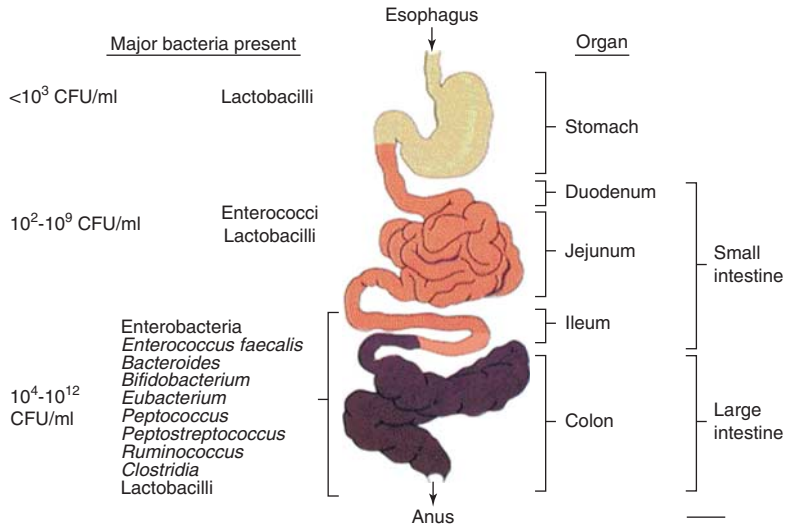

Figure 1. Composition and distribution of human flora in the gastrointestinal tract.

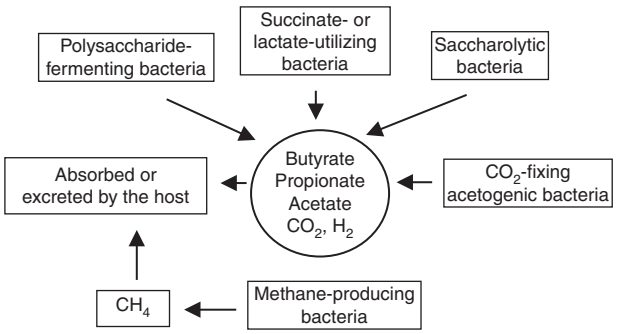

Figure 2. Metabolic activity of intestinal microflora. CH_4, methane; CO_2, carbon dioxide; H_2, hydrogen.

development of high concentrations of methanogens depends on a continuous supply of high H_2 concentrations from exogenous or endogenous sources that exceeds the capacity of removal. The latter processes may be inhibited by physical factors that increase H_2 retention in the large bowel such as diverticulosis, colonic motor disorders, or tumors thus resulting in high concentrations of breath CH_4. It should be pointed out that methanogenesis lowers the pressure that would normally be exerted by a given amount of H_2 because 4 l of H_2 are used to produce 1 l of CH_4 (22).

Methanobrevibacter smithii, which uses H_2 to reduce CO_2 to CH_4, is responsible for almost all CH_4 produced in the intestine (25–27). Another methanogen that contributes to CH_4 production is Methanobacterium ruminatum; moreover, specific species of Bacteroides and Clostridium that reside in the gut can also release CH_4 (28). Non-methanogenic bacterial populations produce acetic, propionic, and butyric acids and also H_2, which is used by *M. Smithii*, confirming that methanogen growth and CH_4 production mainly depends on H_2 produced from endogenous substrates (29).

CH_4 PRODUCTION AND INTESTINAL DISEASES

Colon-rectal cancer

Colon cancer is the third most frequent type of cancer in the industrialized Western countries and remains the second leading cause of cancer death in the United States (30,31). About 5% of all colon tumors is represented by hereditary forms, whereas the majority is characterized by sporadic forms. Western diets rich in animal products including fat, cholesterol, and protein were shown to have carcinogenic properties in experimental studies. The composition of the diet not only influences the quality of gut flora but also helps establish predictable and competitive relationships between the host bacteria (32,33). Several studies have shown that a diet rich in fats and meat but poor in vegetables and fruits promotes the growth of anaerobic putrefactive flora (bacteroides, clostridia, and sulfate reducing bacteria [SRB]) having a pro-carcinogenic effect, and decreases the fermentative flora (lactobacilli, bifidobacteria), having an anti-carcinogenic effect (32,34–36).

At present, there are different theories about the relationship between CH_4 production and colorectal cancer but with discordant results (**Table 1**).

Haines *et al.* reported that CH_4 excretion occurred twice as commonly in patients with colonic cancer compared with the general population, suggesting a difference in the anaerobic intestinal flora. They suggested that colorectal cancer may be caused by cocarcinogens formed as the result of nuclear bile acid dehydrogenation in the large intestine by anaerobic bacteria. International comparisons have shown that stools of individuals in countries with a high incidence of colorectal cancer contain higher fecal bile acid concentrations and larger numbers of anaerobic organisms than the stools of people in low-incidence countries. If an intestinal environment rich in anaerobics enhances the production of cocarcinogens from bile acids, those subjects who produce CH_4 may be more likely to develop colorectal cancer (37).

In a report, CH_4 excretion was also common in patients with colonic disorders generally accepted as premalignant, as extensive

Table 1. Correlation between CH_4 excretion and CRC

Author	CRC and CH_4-ex correlation	CH_4-ex in CRC	CH_4-ex in benign lesions	CH_4-ex in control	CRC in CH_4-ex	CRC in non CH_4-ex	Explanation
Haines (37)	Yes	80%	39%	40%			
Piquè (38)	Yes	91.4%	41.3%	42.9%			
Karlin (39)	Yes	29%		2%			
Gold (40)	No						Tumor-produced mucoproteins
Piquè (38)	No						Tumor delayed transit
Gibson (8)	No						PH dependent
Newmark (41)	No						PH dependent
Thornton (42)	No						PH dependent
O'Keefe (43)	No						Anticarcinogen effect
Hoff (44)	No				5%	5%	
Le Marchand (45)	No						
Sivertsen (46)	No	63%		56%			
Kashtan (47)	No	37.8%		25.4%			
Karlin (48)	No						

CRC, colon-rectal cancer; CH_4, methane; ex, excretors.

ulcerative colitis and polyposis syndromes, but the number of patients studied was small. In contrast, the incidence of CH_4 excretion in patients with ulcerative proctosigmoiditis and benign colonic diseases did not exceed that of the general population (38).

Karlin *et al.* explored the use of fecal skatole and indole and breath CH_4 and H_2 as metabolic markers of the anaerobic colonic flora in patients with unresected large bowel cancer or polyps. Patients with descending or sigmoid colon cancer were more likely to be breath CH_4 excretors than control subjects. Control subjects excreting breath CH_4 excreted less fecal skatole than breath CH_4 excretors in the following groups: patients with adenomatous polyps, colorectal cancer, proximal colon cancer, descending and sigmoid colon cancer, and rectal cancer. These data suggest that fecal skatole excretion ≥100 μg/g feces might be useful to discriminate colorectal cancer patients from control subjects. At present, the explanation of a correlation between colon cancer and CH_4 excretion is still unknown: we do not know whether the methanogenic flora predates the tumor or derives from the tumor. In this respect, different hypotheses have been proposed by several researchers.

The findings might be explained by the presence of a substrate in the tumor from which the anaerobic colonic flora of the colon could produce CH_4. The nature of such substrates is unknown, but the possibility that blood leaking from the tumor is the substrate responsible for CH_4 production seems to have been ruled out. Heme is a growth factor for some anaerobic organisms, but luminal hemoglobin available to colonic microflora in the presence of tumor does not seem to influence CH_4 production.

Growth of methanogens and CH_4 formation require substrate, mainly carbohydrates and proteins. These substrates are of dietary or endogenous origin, but dietary influences on CH_4 excretion have not consistently been demonstrated. CH_4 excretion occurs in fasting and may result from catabolism of endogenous glycoproteins and proteins (39).

Gold and Miller have shown that colonic mucoprotein antigen, the major secretory product of large bowel epithelium, shows increased protein-to-carbohydrate ratio when secreted by the tumor. Therefore, it is possible that changes in the composition of colonic secretions occurring with the tumor development provide preferential substrates for CH_4 production. Alternatively, it is possible that many colonic organisms adhere to the intestinal secretions and mucosa by sugar-specific lectinlike mechanisms. Thus, tumor-induced changes in binding sites may favor methanogen colonization (40).

Partial colonic obstruction by the tumor, causing delayed transit of large bowel contents, could be an indirect cause of CH_4 formation, because it may enhance anaerobic conditions favoring the growth of CH_4-producing bacteria prolonging the exposure time of the hypothetical substrates to the methanogenic flora (38).

Studies of single methanogenic strains demonstrated that CH_4 production is sharply pH dependent, decreasing rapidly at $pH < 7$, whereas other methanogenic strains have a pH optimum of 8. A neutral or slightly alkaline colonic environment may therefore favor methanogenesis (8). Higher fecal pH has been demonstrated in populations at high risk for cancer compared with low-risk controls; preliminary data indicate the presence of alkaline fecal pH in patients who have developed colonic cancer (41). Thornton (42) has suggested that high colonic pH promotes cocarcinogen formation by bacterial degradation of bile acids or cholesterol. Therefore, high colonic pH may favor both methanogenesis and carcinogen formation.

On the contrary, other several interesting studies did not find any difference in CH_4 excretion between patients with cancer and healthy controls.

Native black Africans, who are known to have one of the lowest rates of colon cancer in the world, have been shown to have high levels of methanogens. South African whites consume three times more meat than native black Africans. The significance of this is thought to be the amount of sulfate proteins contained in meat. Sulfate released from these proteins provides substrate for SRB, with production of sulfide that was shown to be very carcinogen for colon mucosa. On the contrary, the methanogenic flora, which competes with SRB for H_2 gas, produces an anticarcinogenic effect (43).

Hoff *et al.*, in an interesting study, evaluated breath CH_4 excretion in relation to colorectal adenomas and possible cancer risk factors. They demonstrated that there was no association between breath CH_4 and prevalence of small or large polyps, of their multiplicity or localization. They concluded that breath CH_4 detection cannot be used as a screening method for the identification of average-risk individuals with precancerous colorectal lesions (44).

Furthermore, there was no difference in the occurrence of colorectal cancer among close relatives of CH_4 excretors compared with the non-excretors. This suggests that the increased risk of colorectal cancer through family disposition is not associated with large-intestinal colonization of methanogenic bacteria. The same results were obtained by other studies *in vivo* and *in vitro* (45–48).

Diverticulosis

The common risk factors for diverticulosis are a diet poor in fiber intake or rich in fat, increasing age, constipation, connective tissue disorders, which may cause colon wall weakness (such as Marfan's syndrome) (49).

Different studies by various researchers have investigated the relationship between CH_4 production and intestinal diverticulosis. In particular, Weaver *et al.* (22) have discovered high methanogen concentrations ($\geq 10^7$/g dry weight of feces) in subjects with diverticulosis compared with controls (58% vs. 25%, respectively); in fact, the diverticula may provide a particularly suitable environment for the growth of methanogens. This could be due to the entrapment of H_2 gas and preferential conversion to CH_4 as opposed to the loss of H_2 in flatus. On the other hand, the diverticula may provide a sheltered niche where the slow growing methanogens are not swept away and where symbiotic relationships with H_2-producing organisms may occur.

Bond *et al.* indicated that CH_4 production occurs primarily in the left colon whereas H_2 is produced primarily in the right colon. As diverticulosis is primarily a left-sided colonic disorder, H_2 accumulation in the diverticula could result in increased methanogenic growth in the left colon with increased methanogenesis and decreased H_2 loss in flatus. Alternatively, H_2 produced in the left colon may be rapidly converted to CH_4 (21).

Inflammatory bowel diseases

Inflammatory bowel diseases (IBDs) (Crohn's disease and ulcerative colitis) are a group of multifactorial inflammatory conditions with genetic and environmental contributions. Some studies have evaluated the correlation between IBD and CH_4 production, but the results are controversial.

McKay (16) evaluated CH_4 excretion in unresected colonic carcinoma, Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS), pneumatosis cystoides intestinalis, and diarrhea of various etiology. Patients with Crohn's disease, ulcerative colitis, and pneumatosis cystoides intestinalis had a significantly decreased prevalence of CH_4 excretion compared with non-gastrointestinal patients. CH_4 prevalence and concentration in patients with Crohn's disease and ulcerative colitis were independent of the disease distribution.

The absence of CH_4 excretion in IBD and pneumatosis cystoides intestinalis may result from an altered epithelial mucosa. The analysis of colonic gas showed a lack of CH_4 production in patients with IBD; several patients with no detectable CH_4 in the breath had low concentrations in the colonic gas. Patients with IBD may lack the capacity for significant CH_4 production or excretion, which may due to differences in relative oxygen tension, blood flow, or membrane condition and permeability.

In another study, Pimentel *et al.* have evaluated whether the different gas pattern on lactulose breath testing coincide with diarrhea and constipation symptoms in IBS and IBD. There was a significantly higher proportion of breath CH_4 excretion during lactulose breath test among subjects with constipation compared to than those with diarrhea. CH_4 excretion among subjects with SIBO and IBS was associated with higher constipation severity scores and lower diarrhea severity scores, as well as with the constipation predominant subgroup of IBS. By contrast, CH_4 excretion was infrequent in diarrhea-predominant IBS and virtually absent in IBD. It is possible that the lower prevalence of CH_4 excretion in IBD and diarrhea-variant IBS may be an artifact of colonic purging. In fact, theoretically, diarrhea may inhibit proliferation of methanogenic bacteria. In support for this hypothesis, colonic lavage can reduce and even eliminate CH_4 excretion for long periods of time (50).

Castiglione *et al.* showed that the prevalence of bacterial overgrowth and the orocecal transit time were higher in patients with Crohn's disease undergoing previous surgery (ileo-colic resection) than in non-operated patients and in controls. Moreover, the overall prevalence of CH_4 producers was very small in the study subjects, indicating that, in this gastrointestinal disease, the proportion of methanogenic bacteria is small (51).

A low incidence of methanogens in IBD has been also demonstrated by a group of microbiologists using mcrA analysis (52).

Irritable bowel syndrome

IBS is a common gastrointestinal disorder, seen in >15% of the population, it is characterized by the following symptoms: diarrhea or constipation, abdominal pain, bloating, sense of incomplete evacuation, straining, urgency, abdominal distension (53,54). Despite this high prevalence and much research interest, the cause of IBS remains unknown. Studies have shown altered gut motility, peripheral and central sensory dysfunction, as well as an exaggerated response to stress. However, there is no finding that can be identified in a majority of patients, and there is

no specific diagnostic test. The Rome criteria, help diagnose and categorize the syndrome (55,56).

Combined sugar malabsorption patterns are common in functional bowel disorders and in most patients may contribute to the symptomatology (57).

Recent evidence suggests that subjects with IBS may have a quantitative or qualitative alteration in gastrointestinal flora with an abnormal colonic fermentation. In fact, the rates of gas excretion (mainly H_2) are much greater in IBS patients than in controls. Moreover, in patients with IBS, an exclusion diet (meat and fish, dairy products replaced by soya products, any cereals other than rice) reduces gas excretion (H_2 and CH_4) and significantly improves symptoms; however, this change was not observed in controls (58,59).

Other findings suggest that IBS patients have excessive small intestinal bacterial overgrowth (60–63).

Follow-up studies in this area are showing associative factors between gut bacteria and IBS that may explain the different types of IBS. The best example is the finding that methanogenic organisms in IBS patients are always associated with constipation-predominant IBS; it seems that CH_4 gas emitted during intestinal fermentation may impact on gut motility (62). This association derives from comparison between the presence of CH_4 and subjective symptoms and objective stool diaries as tested by Chatterjee *et al.* (64).

El Oufir and collaborators showed that mean transit time is inversely related to fecal weight, counts of SRB, total short chain fatty acids concentrations, and H_2 excreted in breath after lactulose ingestion. Conversely, transit time was positively related to fecal PH and tended to be related to methanogen counts. Whether this changes in fecal flora and colonic fermentation primarily result from transit variations or are secondary to the consequences of transit variations needs further discussion (65).

Soares *et al.* (66) showed that colonic transit time is significantly more prolonged in CH_4 producers than in non-CH_4 producers; most importantly they observed that children CH_4 producers with constipation and soiling are more numerous than children CH_4 producers but with constipation without soiling.

In the past few years, the researchers attention has been focused on the relationship between serum serotonin levels and IBS. In all, 95% of all serotonin secreted by enterochomaffin cells is found in the gastrointestinal tract and it causes peristaltic gut stimulation. Recent findings have evidenced that IBS subjects predominantly with diarrhea have elevated postprandial serotonin compared with controls. Pimentel *et al.* have investigated the role of CH_4 in serotonin response and bowel symptoms in patients with IBS. They have shown that baseline serotonin levels are not different between CH_4 and non-CH_4 but they are lower in H_2-producing subjects. Sixty minutes after carbohydrate administration, there was a significantly lower serum serotonin concentration in CH_4-producing IBS subjects compared with H_2. It seems that CH_4 portends a lower postprandial serotonin response compared with that of H_2-producing IBS subjects and may be linked to the finding of constipation among CH_4-producing IBS subjects (67).

Pimentel *et al.* have also shown that CH_4 slows intestinal transit and augments small intestinal contractile activity. They have observed that these contractions are isolated, segmental, and non-propagating (68). In IBS patients, these alterations could be responsible, at least in part, for the onset of gastrointestinal symptoms, such as abdominal pain (**Table 2**).

Table 2. Correlation between CH_4 excretion and IBS

Author	
Pimentel (60)	CH_4 excretion associated with constipation-predominant IBS
Chatterjee (64)	CH_4 excretion associated with constipation-predominant IBS
El Oufir (65)	OCTT is related to methanogen counts
Soares (66)	OCTT is significantly prolonged in CH_4 producers than in non CH_4 producers Children CH_4 producers with constipation and soiling are more than without soiling
Pimentel (67)	Postprandial serum serotonin level in CH_4-producing IBS subjects is lower compared to that of H_2-producing IBS subjects
Pimentel (68)	CH_4 slows intestinal transit and augments small intestinal activity. These contractions are isolated, segmental, and non-propagating

CH_4, methane; IBS, irritable bowel syndrome; OCTT, oro-cecal transit time.

CONCLUSION

Intestinal CH_4 production is a complex mechanism from specific colonic bacteria, involving the metabolism of other gases, particularly H_2. In about one third of adult healthy individuals, it is possible to detect intestinal CH_4 production with no specific clinical relevance. However, an unbalance of gas metabolism and abnormal CH_4 production have been considered in the pathogenesis of several intestinal disorders, including colon cancer, IBD, IBS, and diverticulosis.

Although the data are still controversial, intestinal gas metabolism certainly represents a very interesting chapter of intestinal pathophysiology. Further investigations should be encouraged to better understand the microbiological characteristics and properties of intestinal bacteria and their complex metabolic activities.

ACKNOWLEDGMENTS

This work was supported by an unrestricted grant provided by Fondazione Ricerca in Medicina.

CONFLICT OF INTEREST

Guarantor of the article: Antonio Gasbarrini, MD.

Specific author contributions: Design of the manuscript: Davide Roccarina and Ernesto Cristiano Lauritano; literature review and drafting of the paper: Davide Roccarina and Ernesto Cristiano Lauritano; revision of the paper: Veronica Ojetti, Maurizio Gabrielli, and Francesco Franceschi; final version approval: Antonio Gasbarrini; references: Maurizio Gabrielli; English revision: Veronica Ojetti and Francesco Franceschi.

Financial support: None.

Potential competing interests: None.

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